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07/764,073	9/23/91	ANSON et al	604-8

EXAMINER	
STEPHEN WALSH	
ART UNIT	PAPER NUMBER
1814	38

DATE MAILED:

EXAMINER INTERVIEW SUMMARY RECORD

All participants (applicant, applicant's representative, PTO personnel):

(1) LEONARD C. MITCHARD (3) _____

(2) STEPHEN WALSH (4) _____

Date of interview 5-6-92

Type: ☒ Telephonic ☐ Personal (copy is given to ☐ applicant ☐ applicant's representative).

Exhibit shown or demonstration conducted: ☐ Yes ☒ No. If yes, brief description: _____

Agreement ☐ was reached with respect to some or all of the claims in question. ☒ was not reached.

Claims discussed: 33 and 35

Identification of prior art discussed: Wasley et al — Examiner requested copy be faxed because original copy is missing from file.

Description of the general nature of what was agreed to if an agreement was reached, or any other comments: Examiner proposed insertion of --human-- to obviate potential issue of contamination by fetal calf serum constituents being considered, contamination by plasma constituents; authorization for the amendment as attached.

(A fuller description, if necessary, and a copy of the amendments, if available, which the examiner agreed would render the claims allowable must be attached. Also, where no copy of the amendments which would render the claims allowable is available, a summary thereof must be attached.)

Unless the paragraphs below have been checked to indicate to the contrary, A FORMAL WRITTEN RESPONSE TO THE LAST OFFICE ACTION IS NOT WAIVED AND MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW (e.g., items 1–7 on the reverse side of this form). If a response to the last Office action has already been filed, then applicant is given one month from this interview date to provide a statement of the substance of the interview.

☒ It is not necessary for applicant to provide a separate record of the substance of the interview.

☒ Since the examiner's interview summary above (including any attachments) reflects a complete response to each of the objections, rejections and requirements that may be present in the last Office action, and since the claims are now allowable, this completed form is considered to fulfill the response requirements of the last Office action.

Stephen Walsh
Examiner's Signature

PTOL-413 (REV. 1-84)

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• MESSAGE

Here is a copy of the Wasley et al reference.

Len Mitchard

AS

COAGULATION

1256

EXPRESSION, PURIFICATION AND CHARACTERIZATION OF BIOLOGICALLY ACTIVE HUMAN FACTOR IX SYNTHESIZED BY RECOMBINANT MAMMALIAN HOST CELLS. C.Shoemaker*, L.Vanlar*, R.C.Furie, B.Furie, and R.Kaufman*. Genetics Institute, Cambridge MA and Tufts-New England Medical Center, Boston MA.

Factor IX has been expressed to high levels within a recombinant host cell and subsequently purified to homogeneity for characterization. Coding sequence for full-length factor IX was inserted into a mammalian cell expression vector and transfected into Chinese hamster ovary cells. The integrated DNA was amplified to high copy number by selection for increasingly higher expression levels of a marker gene, dihydrofolate reductase, contained within the plasmid. Thus far, cloned cell lines secreting over 100 µg/ml of Factor IX antigen and up to 1.5 µg/ml of native Factor IX antigen have been obtained. Expression of biologically active factor IX is dependent on the presence of vitamin K in the media. The γ-carboxylated Factor IX was isolated from cell culture fluid (21 µg/ml Factor IX antigen; 0.8 µg/ml native Factor IX antigen) by immunoaffinity chromatography using antibodies conformation-specific for the metal-stabilized conformer of Factor IX. This conformation is dependent upon metal ions and γ-carboxyglutamic acid. Purified recombinant Factor IX migrated as a single band on SDS PAGE with an electrophoretic mobility equivalent to plasma-derived Factor IX. The purified material demonstrated Factor IX coagulant activity, in Factor IX-deficient plasma, with a specific activity of 40 U/mg. Amino acid analysis of the alkaline hydrolysate of recombinant Factor IX demonstrated the presence of γ-carboxyglutamic acid. Immunoassays of recombinant Factor IX with anti-Factor IX antibodies and with conformation-specific anti-Factor IX:Ca(II) antibodies measured equivalent Factor IX levels. Current efforts are directed at improving the efficiency of γ-carboxylation and increasing the percentage of Factor IX that is biologically active. Isolation of recombinant Factor IX and direct demonstration of the presence of γ-carboxyglutamic acid and Factor IX coagulant activity in the purified recombinant Factor IX indicate the potential for preparation of Factor IX by recombinant DNA technology for treatment of hemophilia B.

1257

INTERACTIONS BETWEEN CANCER CELL TISSUE FACTOR, PLATELETS & THE PLASMA COAGULATION SYSTEM:POTENTIAL FACTORS FOR METASTASIS. J.H.Silberberg*, D.Wilkie*, S.Zucker, VA Medical Center Northport, NY and SUNY @ Stony Brook.

The activation of platelets and the plasma coagulation system has been proposed to play an important role in cancer metastasis. We have evaluated the interaction of these factors in four highly metastatic human cancer cell lines derived by xenogenic transplantation (pancreatic ductal lines RWP1 and 2, small cell lung cancer lines H-69 & 128). The H-69 line showed minimal procoagulant activity & no aggregation of heparinized platelet rich plasma (PRP). The other three lines substantially shortened recalcification times in normal plasma and XII deficient plasma but not in VII and X deficient plasmas. All three lines released this activity into the supernatant where it could be pelleted at 100,000g for 2 hours. All three of these lines aggregated heparinized(4u/ml)PRP with release of ATP after a lag period which varied with the concentration of tumor material used. Heparin concentrations of 40u/ml in PRP completely inhibited platelet aggregation. To localize procoagulant activity in cells, RWP1 and 2 lines were both disrupted by nitrogen cavitation with isolation of cell organelles by sucrose density gradient centrifugation. Results:Aggregation and shortening of recalcification times occurred primarily in the plasma membrane enriched fractions. Tumor cytosol minimally shortened recalcification times but had no effect on aggregations. Inhibitors of serine proteases (DFP,PMSF) and EDTA did not affect either recalcification times or aggregation. Monoclonal antibodies to human brain tissue factor [illegible] used to characterize tumor

1258

AUTOACTIVATION OF HUMAN FACTOR XII(HAG OF HEPARIN AND LOW MOLECULAR WEIGHT DE Michael Silverberg and Susan Vest Dieh Allergy, Rheumatology and Clinical Imm Sciences Center, SUNY, Stony Brook, N.

Factor XII undergoes autoactivatior negatively charged surfaces. Particul high molecular weight dextran sulphate activators but the effect of molecular reaction has not previously been e sulphate of 5,000 MW, contain.ng 40% was able to support the autoactivatio an apparent rate constant 1/5 that of sulphate of similar sulphate content. sulphate was treated with o-phenanthr incubated with Factor XII in the pres efficiency of autoactivation was unch formation of high molecular weight co metal ions is not required for activa weight compounds. The 5,000 MW dextr analysed by gel filtration on Sephad fractions tested for their ability to activation of Factor XII; the apparen activation was dependent upon elutio over a 6-fold range with decreasing different Heparin preparations were USP Heparin, the others were of nomi 13-15,000. All supported the autoact Chromatography on G-75 yielded diffe all of the plots of apparent rate co volume were superimposable and showe 8-fold decline across the peak. The both classes of sulphated polysacch factor XII can interact with even l polyanions to generate active enzym the activation is dependent upon th activator and declines markedly at approximately 12,000 MW.

1259

KINETIC STUDIES USING MONOCLONAL AN AN ENZYME-SUBSTRATE BINDING SITE FO HEAVY CHAIN OF FACTOR XIa. D. Sinh R.W. Babilon,* and P.N. Walsh, Thro Temple University School of Medicin

The heavy chain of human coagula in addition to the active-site cont essential for calcium-dependent act substrate factor IX (FIX). In orde the heavy chain of FXIa possesses while its catalytic site resides on studied the kinetics of the activa two monoclonal antibodies, one (SP4 light chain of FXIa and the other (chain. Analysis of the kinetic da plots of 1/V vs. 1/S at various co light chain specific antibody SP4 in the value of Vmax as the concent was increased whereas the value of an example of classical noncompeti the binding of SP4 to the light ch torts the enzyme sufficiently to p tioning of the catalytic center, t nonproductive. In contrast, in th concentrations of the heavy chain the Vmax remained unchanged, where crease in the value of the apparen was observed: an example of class tion. Thus, the binding of the an chain domain of FXIa causes a chan of the enzyme that distorts the su [illegible] the substrate fro